California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Monitoring Section
3292 Meadowview Road
Sacramento, CA 95832

DETERMINATION OF THIRAM IN SURFACE WATER

1. **Scope:**
This method is for the extraction and analysis of thiram in surface water and is followed by all authorized personnel of the Environmental Monitoring Section.

2. **Principle:**
Thiram is extracted from surface water samples by liquid-liquid extraction with methylene chloride. The extract is concentrated on a rotary evaporator, solvent-exchanged into methanol on an N-evaporator, and analyzed by LC/UV.

3. **Safety:**
Extractions should be conducted in a hood. Safety glasses and gloves should be worn when handling solvents and chemicals.

4. **Interferences:**
No interferences were observed during method validation. Matrix interference may occur in some samples. Recovery may be reduced if an emulsion forms during the extraction.

5. **Apparatus and Equipment:**
   - 5.1 2 lieter separatory funnels, Kimax or equivalent
   - 5.2 500 milliliter boiling flasks, Kimax or equivalent
   - 5.3 15 milliliter calibrated centrifuge tubes, Kimax or equivalent
   - 5.4 Miscellaneous glassware as required
   - 5.5 Sample rotator capable of rotating at approximately 30 revolutions per minute
   - 5.6 Rotoevaporator, Büchi or equivalent
   - 5.7 Nitrogen evaporator (N-evap), Meyer Organomation, or equivalent
   - 5.8 Liquid chromatograph equipped with autosampler and uv detector, column, temperature setting, mobile phase and flow rate appropriate for compound being analyzed, Waters Alliance or equivalent

6. **Reagents and Supplies:**
   - 6.1 Methylene chloride, Fisher pesticide grade or equivalent
   - 6.2 Methanol, Fisher HPLC grade or equivalent
   - 6.3 Acetonitrile, Fisher HPLC grade or equivalent
   - 6.4 Water, Fisher HPLC grade or equivalent
   - 6.5 Analytical thiram standards
     - 6.5.1 Stock standard (100.0 ug/ml)
     - 6.5.2 Working standard (10.0 ug/ml)

7. **Standard Preparation:**
   - 7.1 Use clean volumetric Class A glassware. Manufacturer's certification of volume is acceptable for calibration.
   - 7.2 Obtain standard stock solution from CDFA CAC Standards Repository.

7.3 Allow standard stock solution and solvents to reach ambient temperature.

7.4 For the working stock standard preparation, prepare a 10X dilution of the Repository stock solution at 100.0 ug/ml. Using a volumetric pipette or microliter syringe, transfer the calculated amount of standard stock solution into an appropriately sized volumetric flask. Bring to volume with methanol, and mix thoroughly. Because the stability of thiram standards has not been fully characterized, no more than 25 ml of the 10.0 ug/ml working standard solution should be prepared at one time. Transfer the solution to an appropriate container.

7.6 Label, using ink and smear proof label. Show the following:

- 7.6.1 Chemical name
- 7.6.2 Concentration
- 7.6.3 Date prepared
- 7.6.4 Solvent used and lot number
- 7.6.5 Initials of person preparing standard
- 7.6.6 Study reference if appropriate
- 7.6.7 Expiration date: 2 months from preparation date or same as stock, if sooner
- 7.6.8 CAS # and Standards Repository #

8. **Sample Preservation and Storage:**

Water samples must be extracted within 24 hours of collection. Unextracted samples are stored in a refrigerator at 3° C ± 3° C.

9. **Test Sample Preparation:**

9.1 Liquid-liquid extraction

- 9.1.1 Remove samples from the refrigerator and allow to come to room temperature.
- 9.1.2 Weigh the sample jars, and transfer 800 ± 20 ml to a 2 liter separatory funnel. Reweigh the sample jar with the remaining sample , and record the sample weight obtained by difference to one decimal point.
- 9.1.3 Add 100 ± 10 ml methylene chloride to the separatory funnel, and shake vigorously for one minute, venting as needed.
- 9.1.4 Allow the phases to separate. If a persistent emulsion is formed, add sodium chloride or sonicate the flask to break the emulsion.
- 9.1.5 Drain the lower methyene chloride later into a 500 ml round-bottom flask. (Note that the extract is **not** filtered through sodium sulfate.)
- 9.1.6 Repeat steps 9.1.3 – 9.1.5 twice with 80 C aliquots of methylene chloride.
- 9.1.7 Reduce the combined extract on a rotary evaporator to 5-10 ml using a water bath temperature of 30-35 °C. **Do not exceed a temperature of 35 °C. Do not reduce the sample volume to less than 5 ml. Higher temperatures or excessive evaporation will reduce thiram recovery.**
- 9.1.8 Quantitatively transfer the concentrated extract to a calibrated centrifuge tube with methylene chloride.

9.1.9 Evaporate the sample to 1-1.5 ml on a nitrogen evaporator using a **gentle** stream of nitrogen using a water bath temperature of 30-35 °C. Do not allow the sample to splash in the tube. Do not exceed a temperature of 35 °C.

9.1.10 Add 1.5 ± 0.2 ml methanol to the tube, and vortex to mix.

9.1.11 Again evaporate the sample to 1-1.5 ml.

9.1.12 Add 1.5 ± 0.2 ml methanol to the tube and vortex to mix.

9.1.13 Evaporate the sample to <1.5 ml. Adjust the final volume to 2.0 ml according to the calibration of the tube with methanol. Vortex to mix and divide the final extract among three autosampler vials. Use one vial for analysis, and store the others in a sample refrigerator for retention according to the study protocol.

10. **Calibration standard preparation:**

10.1 The following procedure was used for the method validation. If the stability of diluted standard solutions is demonstrated, this procedure may be modified.

10.2 Calibration standard solutions shall be prepared to cover the concentration range of interest. The following levels are recommended: 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, and 5.0 ug/ml. These solutions can be prepared in autosampler vials using 100 and 500 µl syringes. The three highest concentrations are prepared by dilution with methanol of the 10.0 ug/ml stock standard solution by factors of 10, 5, and 2. The lower concentrations are prepared by dilution of a portion of the 1.0 ug/ml standard with the appropriate amount of methanol.

10.3 The number of calibration standard solutions and the concentrations used may be changed according to the study protocol and the analyst's judgment. A minimum of three calibration levels shall be used.

11. **Instrument calibration:**

11.1 Analyze one or more standard curves on appropriate instrument using analytical standards. A standard curve consists of a minimum of three levels and brackets the anticipated sample levels of the compound being analyzed or covers the linear range of the instrument.

11.2 Acceptance criteria for linearity are $r \geq 0.995$ and a maximum quantitative error of 15% or less between a smooth curve and the point-to-point line, measured at the midpoint between consecutive standard levels. Acceptance criteria for reproduciblity between standards curves is 15%.

12. **Analysis:**

12.1 Limit of detection (LOD) is based on at least a 3:1 signal to noise ratio, as measured on a clear section of the baseline adjacent to the peak of interest. Limit of quantitation (LOQ) is based on a minimum 10:1 signal to noise ratio or 3.33 times the LOD.

12.2 Analyze one matrix blank and one matrix spike per sample set of up to 20 samples. Use the matrix water and spiking level specified in the study protocol. For the recommended spiking level of 5.0 ppb, add 400 µl of the 10.0 ug/ml stock standard solution to an 800 ml sample of the matrix

water and mix thoroughly before extraction. (The final extract volume for this spike level should be 4.0 ml for a response in the middle of the calibration range. A recovery of 100% of a 5 ppb spike in a final volume of 4 ml is equivalent to a 1.0 ug/ml standard concentration.) Recoveries between 60% and 120% are acceptable.

12.3 Sample sets are bracketted by standard curves and are quantitated against the first curve. Acceptance criterion for linearity is $r \geq 0.995$.

13. **Analysis:**

13.1 Injection Scheme

The instrument may need to be conditioned with a matrix blank or old sample before running the following sequence: Instrument blank (methanol), Standard Curve, Instrument blank (methanol), Method Blank, Matrix Spike, Test Samples (maximum of 9, with duplicate injections) and Standard Curve.

13.2 Instrumentation

13.2.1 Analyze thiram samples using a gas chromatograph equipped with a dual wavelength UV detector. (A diode array detector may be used if it has sufficient sensitivity to achieve the specified reporting limit.) The primary analytical wavelength is 275 nm. The response of the second analytical wavementh at 290 nm should be within ± 15% of the primary response. A greater difference may indicate a co-eluting interference.

13.2.2 This method was validated using a Waters Alliance LC system consisting of a 2695 Separation Module, a 2487 dual wavelength detector, and an Empower 2 data system. An equivalent LC system may be used after appropriate demonstration of system suitability.

13.2.3 Recommended instrument parameters for the Alliance system: Column: Zorbax SB C8 15 X 4.5 mm; Column temperature 35 ± 2.5 °C; injection volume 20 uL; wavelengths 275 and 290 nm; time constant 1 sec, sampling rate 1 pt/sec, Hamming filter.

13.2.4 The following acetonitrile-water gradient was used for validation of this method. This gradient may be adjusted according to the analyst's judgment if a different column is used, or interferences arise.

Time 0.0 min: 70% water/30% acetonitrile
Time 1.0 min: 70% water/30% acetonitrile
Time 5.0 min: 10% water/90% acetonitrile
Time 8.0 min: 10% water/90% acetonitrile
Time 8.5 min: 70% water/30% acetonitrile
Time 10.0 min: 70% water/30% acetonitrile

Flow rate is 1.0 ml/min

Thiram retention time ~6.6 min

14. **QC/QA:**

14.1 Limit of detection (LOD) is based on at least a 3:1 signal to noise ratio, as measured on a clear section of the baseline adjacent to the peak of interest. Limit of quantitation (LOQ) is based on a minimum 10:1 signal to noise ratio or 3.33 times the LOD.

14.2 Analyze one matrix blank and one matrix spike per sample set of up to 20 samples. Use the matrix water and spiking level specified in the study protocol. For the recommended spiking level of 5.0 ppb, add 400 µl of the 10.0 ug/ml stock standard solution to and 800 ml sample of the matrix water and mix thoroughly before extraction. Recoveries between 60% and 120% are acceptable.

14.3 Sample sets are bracketted by standard curves and are quantitated against the first curve. Acceptance criterion for linearity is $r \geq 0.995$.

15. **Calculations:**

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The software uses a linear curve fit, with all levels weighted equally. Alternatively, at the chemist's discretion, concentrations may be calculated using the response factor for the standard whose value is < 30% to the level in the sample.

$$ppb = \frac{(\text{sample peak area}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample})(1000\ \mu L/mL) \times 1000}{(\text{std.peak area}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

16. **Discussion and References:**

16.1 No sodium sulfate is used to filter or dry the sample extracts.

16.2 The sensitivity of this method appears to be limited by the extraction efficiency of thiram from water. Below 5 ppb, recoveries were found to be highly variable. Therefore, the limit of quantitation (LOQ) for the method is set at 5.0 ppb, even though much lower equivalent concentrations of thiram standard can be detected by the system. The sensitivity/precision cited in the earlier method of Paul Lee et al could not be reproduced.

16.3 The instrument response was highly linear for calibration standards from 0.05 to 5.0 ug/ml (correlation coefficient >0.999). The calibration range could be extended to higher concentrations if desired.

17. **References:**

Environmental Monitoring Section Method "Determination of Thiram in Water", Paul Lee, 8/3/1994.

APPROVALS

Revised By:

Pamela Fitch
Staff Chemist

6/30/06
Date

Approved By:

Elaine Wong
Program Supervisor

6/30/06
Date

Approved By:

Balwinder Sekhor
Quality Assurance Officer

Date

Revision Log

Date	What was revised? Why?
6-30-06	Revised method rewritten to conform to Branch format
	Sodium sulfate eliminated (Section 9) and solvent exchange procedure (Section 9.1.9 – 9.1.13) modified to inprove method performance.

THIRAM VALIDATION - RAW DATA (PEAK AREA)

STD CONC (ug/ml)	6/16/06 EXTR SET start	6/16/06 EXTR SET end	6/19/06 EXTR SET start	6/19/06 EXTR SET end	6/20/06 EXTR SET start	6/20/06 EXTR SET end
0.05	2651	3071	2359	2922	2370	2551
0.10	4974	4835	4879	5648	5417	5406
0.25	14484	13859	13800	13568	13765	14025
0.50	28017	27728	26528	26681	27127	26869
1.00	56696	56274	55161	55241	57174	56992
2.00	121989	119066	198383*	198383*	122952	124133
5.00	299765	291683	291396	290098	292681	287627

* - outlier not included in calibration

SPIKE	EQUIV EXTR CONC (UG/ML)	INJ 1	INJ 2	INJ 1	INJ 2	INJ 1	INJ 2
Blank		ND	ND	ND	ND	ND	ND
1 ppb	0.4	15346	14657	5475	4499	12623	11522
2 ppb	0.8	32284	33966	46086	34416	27698	27373
5 ppb	1.0	47280	45468	49684	49923	49109	49373
10 ppb	1.0	50980	51956	51369	51999	55321	55321

THIRAM MDL EXTRACTION SET (6/22/06)- RAW DATA (PEAK AREA)

CONC (ug/ml)	MDL EXTR start	MDL EXTR end
0.05	2505	2646
0.10	5318	4890
0.25	13947	13864
0.50	26315	22614
1.00	57520	58280
2.00	121019	122107
5.00	291331	295943

SPIKE	INJ 1	INJ 2
Blank	ND	ND
5 ppb A	45201	46463
5 ppb B	43411	43151
5 ppb C	48423	48534
5 ppb D*	38334	38406
5 ppb E	43818	45223
5 ppb F	40952	42124
5 ppb G	42076	42591
5 ppb H	43935	46416

*NOTE: MDL D went too far on the Roto-vap - not included in calculations

CALIBRATION EQUATIONS FROM NORTHWEST ANALYTICAL SOFTWARE

6/16 EXTR	CONC = 1.659E-05 * PEAK AREA + 0.019
6/19 EXTR	CONC = 1.709E-05 * PEAK AREA + 0.028
6/20 EXTR	CONC = 1.695E-05 * PEAK AREA + 0.009
MDL EXTR	CONC = 1.704E-05 * PEAK AREA + 0.010

THIRAM VALIDATION - CALCULATED RESULTS
(all calculations are for the first injection, CONC in ug/ml for extract)

	EQUIV	6/16/06 EXTR			6/19/06 EXTR			6/20/06 EXTR		
SPIKE	EXTR CONC	PK AREA	CONC	% REC'Y	PK AREA	CONC	% REC'Y	PK AREA	CONC	% REC'Y
Blank		ND		ND	ND		ND	ND		ND
1 ppb	0.4	15346	0.274	68.4%	5475	0.121	30.3%	12623	0.304	76.0%
2 ppb	0.8	32284	0.555	69.3%	46086	0.812	101.5%	27698	0.559	69.9%
5 ppb	1.0	47280	0.803	80.3%	49684	0.873	87.3%	49109	0.922	92.2%
10 ppb	1.0	50980	0.865	86.5%	51369	0.902	90.2%	55321	1.028	102.8%

THIRAM MDL EXTRACTION SET (6/22/06)- RAW DATA (PEAK AREA)

SPIKE	PK AREA	MDL EXTR CONC	% REC'Y
Blank	ND		ND
5 ppb A	45201	0.780	78.0%
5 ppb B	43411	0.750	75.0%
5 ppb C	48423	0.835	83.5%
5 ppb D*	38334	0.663	66.3%
5 ppb E	43818	0.757	75.7%
5 ppb F	40952	0.708	70.8%
5 ppb G	42076	0.727	72.7%
5 ppb H	43935	0.759	75.9%
AVER CONC:		0.727	
STD DEV:		0.040	
MDL:		0.126	

(100% RECOVERY EQUIVALENT TO EXTRACT CONC OF 1.0 UG/ML)

* NOTE: MDL D went too far on the Roto-vap - not included in MDL calculation

MDL = t* S where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

Thiram in Water Project Summary report

SUUMARY: The 1994 Environmental Monitoring method for the determination of thiram in water was run repeatedly. The stated reporting level of 0.2 ppb could not be achieved, or even approached. A number of modifications of the extraction and detection scheme were tried without success.

The method was validated at spiking levels of 5 and 10 ppb in American River water. Recoveries ranged from 80 to 103%. At spiking levels of 1 and 2 ppb, recoveries were highly variable. Thus it appears that the useful limit of quantitation of the method is 5 ppb.

LC/MS-ESI was evaluated as an alternative to the LC/UV detection specified in the method. A validation could not be completed because of instrument problems (possibly the result of excessive temperatures in the laboratory). However, it was demonstrated that LC/MS is both much more sensitive and selective than LC/UV, and could be a preferred alternative. When the instrument was working, a level of 10 ppb could be detected directly in a spiked water sample with no sample preparation. It is possible that this performance could be improved somewhat with more work.

DETAILS: I began the thiram in water project the last week in February when the Standards Repository had obtained neat standard material and prepared ampoules of stock solution.

After reviewing the 1994 Environmental Monitoring method written by Paul Lee, and consulting with Scott Fredrickson, I began with a stirrer bar extraction as done by the Worker Health and Safety group. This approach is simpler and faster than a separatory funnel liquid-liquid extraction. The reporting level of the old method was 0.2 ppb, so spike levels of 0.2 and 0.5 ppb were tested initially. Two trial extractions gave no recovery at either level, so the stirrer bar extraction was abandoned.

The initial tests had established that the instrumental sensitivity of the Agilent 1100 LC/UV system used was much higher than needed to achieve the target reporting level of 0.2 ppb. So the sample volume was reduced to 400 ml and 50 ml aliquots of methylene chloride. This allowed a 1 L separatory funnel to be used instead of a 2 L funnel, and significantly reduced the amount of methylene chloride per sample. However, no recovery of thiram was obtained.

At this point, Holly Chuek ran the Environmental Monitoring method as written, starting with a fresh ampoule of standard from the Repository. Holly was also unsuccessful in getting any thiram recovery.

At the beginning of the project, I met with David Rauser of Phenomenex to discuss a solid phase extraction (SPE) method for thiram. Late in March, he provided a box of polymeric Strata SPE cartridges and suggested a trial method for evaluation.

I switched to the Quantum triple-quadrupole LC/MS system for the analysis of extracts. LC/MS offers a more sensitive and selective detection than LC/UV, and so improves the signal-to-noise ratio for a given concentration. Because thiram is thermally labile, electrospray ionization was used instead of APCI. Thiram does have a distinct and structurally significant fragmentation pattern by electrospray, but its LC/MS sensitivity is low compared to analytes such as the triazine herbicides. I spent some time optimizing the instrumental parameters. To increase the injection volume, the final solvent for the sample extracts was changed to 1:1 methanol:water instead of methanol. With this change, a 50 µl injection could be made, increasing the sensitivity. However, it was found that thiram was less stable in the mixed solvent than in 100% methanol, even with refrigeration. So it was concluded that this was not a practical modification to the method.

Using the triple quad, I could demonstrate that the Phenomenex Strata SPE cartridges absorbed thiram efficiently from a spiked water sample. However, I could not recover the thiram from the cartridge. Susan Griffin also tried the SPE extraction, without success.

In late April, I also consulted with Max Erwine, a technical specialist at Varian about SPE phases in their product line that might work for thiram. I faxed him several literature references I had found. After reviewing these, he concluded that thiram is probably not a good candidate for SPE extraction with current technology.

LC UV?

I then went back to the original methylene chloride liquid-liquid extraction using the volumes given in the 1994 method. The effort to match the stated reporting limit of the 1994 method was abandoned. A range of spiking levels was checked to find the current practical working range of the method. The extraction gave satisfactory thiram recoveries at spiking levels of 5 and 10 ppb, and variable recoveries at 1 and 2 ppb. No recovery could be obtained at 0.5 ppb. I cannot explain the discrepancy with the results reported in 1994.

Installation and initial training on the new Waters Alliance LC/UV system was completed early in June. Since the LC/MS system needed repair (and could not be used in any case since the laboratory air conditioning system was not working properly), the method validation was shifted to the Waters system, which appears to be significantly more sensitive and stable than the available Agilent 1100 LC instruments.

Stability

NOTES: After this project was underway, memos from Paul Lee to Cathy Cooper from 1992 were found. These memos discussed the instability of thiram in water at different pH levels. Literature sources differ on this point. A stability study should be conducted for each matrix water type before analysis of samples.

Thiram does not have a strong or distinctive UV absorption spectrum. This could make it difficult to detect an interference in the analysis. Using LC/MS-ESI as a detector could eliminate this problem. This would require additional method validation work, but would greatly increase the confidence in the identification of the target analyte. Alternatively, LC/MS could be used to confirm positive samples found by LC/UV.

Control of the rotary evaporation step of the sample preparation was found to critical to acceptable recoveries. If the sample is concentrated too far, thiram is lost, either by breakdown or absorption by the flask. The use of a thiram analog such as disulfuram as a recovery surrogate would increase confidence in the analytical results. Again, this would require additional work.

Written by: Pamela Fitch, Staff Chemist
Date: 6/30/06

6/30/06